grow in the presence of NGF. This cannot be said of hormones which stimulate the receptive organs or tissues but are not indispensable for their survival and growth.

A last but not less important difference between hormones and our growth factors is temporal in nature. Hormones display their function rather late in life. Although hormonal effects are already apparent during fœtal life, it is in the post-natal life and in the fully grown organisms that the role of hormones becomes prominent. Our growth factors on the contrary are most important during early growth and differentiation of the target cells. Indeed some of these cells, such as the sensory nerve cells, are receptive to the growth effect of the NGF only during a very restricted and early period of their growth. Even the sympathetic nerve cells, which respond to this agent throughout life, show a maximal growth response during the early phase of their differentiation. The same is true for the epidermal growth factor.

It is tempting to suggest that specific growth factors such as those described might be regarded as a sort of more primitive and fundamental integrative system than hormones. They are possibly metabolites released by cells still not organized in well defined organs and utilized by other cells as growth factors. Since their main function is indeed to promote growth in the responsive cells, the non-committal term of 'growth factors' seems to be appropriate at present, though we should be ready to replace it with a more precise term as our knowledge of these remarkable biological agents gains in precision and depth.

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Size, Growth, and Growth-control in Developing Animals

Introduction

The quite extraordinary phenomenon discovered by Levi-Montalcini & Hamburger (1951) and so successfully explored since by Levi-Montalcini and her colleagues will clearly have to be accommodated within any general theory of growth control. This will be so even if nerve growth factor (NGF) itself proves to have a restricted role in determining normal growth patterns, because the specific effects of anti-NGF serum must surely mean that NGF is an essential, if not necessarily a limiting, factor in normal development. It may therefore be worth referring briefly to some of the more general problems of growth control that developmental biology has so far tackled.

It is, of course, almost axiomatic that whole organisms and many of their parts are capable of growing at rates far in excess of those actually achieved. The decline of specific growth rate with age is not, as Medawar has pointed out, a process with an in-built inevitability to be compared with, say, the increase of entropy in an open system. Indeed, there are many circumstances in which it can be temporarily or locally overcome, as in the catch-up growth of individuals restored to favour after a period of ill-health or malnutrition (*see* e.g. Tanner 1964) or in the proliferation released by the conditions of *in vitro* culture. NGF, in this sense, is not alone. Even those organs which are not demonstrably growing at all, but which do have a low rate of cellular replacement, may have this rate lowered by

growth-controlling factors as shown by Santler (1957) for the thyroid.

Yet if post-natal life gives us reason to suppose that growth performance is less than growth potential, it is in embryonic life that growth begins, is most rapid, and most influential in shaping the structure of the adult. The embryologist also has material which offers unusual opportunities for the experimental analysis of growth processes.

Modes of Growth

Biologists are loath to grant the title of biological growth to any process not involving cellular hypertrophy or hyperplasia. Yet, in principle, a system might grow without change in cell size or in rate of cell replacement. For example, if size is dependent upon a balance between cell death and cell replacement, then a change in the mean expectation of life of the cells in the system will be reflected in a proportional change in its size (*see* Goss 1964 for discussion).

Embryologists are particularly conscious of a mode of growth which, as Abercrombie (1958–9) has pointed out, probably makes little contribution to the growth of adult organs, but can be of enormous importance to embryonic ones. This is cellular recruitment, the subversion of cells which belong to one organ or organism into the substance and service of another. Only rarely, as in Siamese twins or in twins sharing an intrauterine circulation, could this mode actually be the basis for growth of a whole organism. Nevertheless, in experimental conditions it may be of critical importance. It is the basis of much embryonic regulation and must occur during the twinning that follows the division of a blastula into two. It certainly occurs during such metaplastic regenerative processes as the replacement of the crystalline lens from the iris margin in lentectomized newts of the genus *Triturus*. It may also occur to complicate the interpretation of growth phenomena following homologous organ transplantation. Spleens grafted on to the chorioallantoic membrane of chick embryos can contribute cells, by the vascular route, to the spleens of their hosts. Splenomegaly in these circumstances is, even if we ignore immunological interactions, far from a pure growth response by the host.

Initial Size and Final Size of the Whole Organism

It is theoretically possible that an organism's ultimate size, or that of any of its organs, might be achieved by multiplying an original size by a genetically determined number. We need not consider in detail the several ways in which such a genetical instruction might be obeyed because it is easier first to test whether it ever is.

Egg size may vary for genetical or nongenetical reasons. Variations between the eggs produced by different females may be expected to have a genetic component, and indeed racial, intraspecific, differences in egg size have been reported in *Drosophila melanogaster*, in frogs, and in rabbits. However, such differences are not found to be related in any universal way to differences in definitive body weight or in growth rates. This is so despite the finding that high initial growth rate may be associated with a large final size.

But though her genotype may influence both the mean size of the eggs produced by any one female, and the distribution of sizes among them, the eggs themselves (in most species) grow to their definitive pre-fertilization size before meiosis. Differences in size between them will therefore not be due to genetic differences, because they will all – during growth – share the genotype of the female. To this natural, nongenetic, variability in egg-size may be added the various experimental devices at our disposal for increasing or decreasing initial egg size. These can be as simple as dividing eggs into two or more parts before or after cleavage has begun, or as fusing eggs together to form giant embryos. Both these operations have now been accomplished in mammals and the products brought to full term or beyond (Seidel 1952, Tarkowski 1964). Equally ingenious is the study of the development of eggs produced by polyploid female salamanders. These, like other cells in such polyploids (*see below*), are unusually large but after meiosis and fertilization may themselves be diploid.

The answer in all these cases is the same – the original differences in size may persist for a time but where animals live long enough for decision, ultimate body size is not proportional to initial size. This, after all, is what we might expect from experience with monozygotic twins.

However, what is true of the whole organism need not be true of its parts.

Initial Size and Final Size of Organs

At some point in early embryogeny each organ, having received its allocation of cells, becomes sufficiently discrete for its initial size to be measured. We may ask whether this initial allocation is by number of cells or by volume of cells,

since either would lead to the same result in normal circumstances. The use of techniques for varying the ploidy (number of haploid chromosome sets per cell) of fertilized eggs, and hence of their mitotic progeny, has given us one series of answers, and the use of transplantation techniques has taken us further.

Nuclear volume and cell volume in amphibians are roughly proportional to ploidy. Thus haploid cells are half the volume of (normal) diploid ones, triploid cells are 50% larger again, and tetraploid ones are twice the normal volume. However, animals composed entirely of cells of abnormal ploidy are not markedly bigger or smaller than normal ones, especially during early life. Nor do their organs depart greatly in proportion from the normal, either as the initial rudiments or later as functional units. It follows that the original allocation of cells, no less than the control of growth, has been sensitive to total volume of tissue and not to cell number.

That this is a true interpretation and that we have not been deceived by some extreme form of computational skill on the part of the embryo is further suggested by the transplantation of presumptive tissues between embryos of different ploidy. Even in a normal diploid environment haploid ectoderm will be induced by its diploid host to provide a lens of normal size but excessive cell population.

There is, perhaps, one qualification of the cell number and tissue size pattern which should be mentioned. There are, even in vertebrate animals, some structures which are large enough to have anatomical status but whose dimensions are set by the dimensions of their component cells. Thus the length of the embryonic myotome is set by the length of the individual myoblasts. My colleague Dr L Hamilton finds that these are shorter in haploids than in diploids of *Xenopus* in approximately the ratio of 3 to 4. However, the total number of segments, and hence of myotomes, is the same in each. Hence haploid embryos, whose total volume is determined by egg size and thus equals that of diploids, are distorted by having short dorsal surfaces with a compensating bulge of the belly. This example shows that embryos *can* count, but perhaps we need not be surprised that while they can count up to the several dozen needed to deal with body segments, they use volume measurements when unit numbers are very high (*see* Smith 1960).

Experiments may reasonably be based upon the assumption of relative constancy in the initial size of the rudiments of each organ in the embryos of any one species. However, there are interspecific differences in rudiment size, and we may ask whether the partition of the total volume of the embryo between its various organs demands a complex of interactions, e.g. with a competitive element, or is otherwise effected. Interspecific transplantations show that there is no generally true answer to this question. Even in inductive situations the size of the induced structure may sometimes be appropriate for the species from which its cells have come (as is the case with lens) and sometimes may conform to the size appropriate to that of the inducer and its species.

We may also examine the developmental consequences of mechanically reducing the size of an organ rudiment (by partial excision) or increasing its size by grafting in homologous material. Though such experiments frequently reveal great powers of quantitative regulation in developing organs, there are often limits beyond which change in size of a rudiment produces qualitative change in the definitive organ. As Wolff and his colleagues have shown (*see* Wolff 1958), the chick limb-bud will develop normally after minor excisions, or after small doses of ionizing radiation, but will react to larger ones by producing fewer digits than normal rather than the normal number of digits, each small. On the other hand, by increasing the material available for leg development it is possible to produce legs with a fully-formed fibula – a condition not known in normal birds since *Archæornis*, i.e. for more than a hundred million years.

However, the mere capacity to produce a more or less normal definitive size despite some alteration in the starting point for growth, means that growth rates must sooner or later be adjusted to provide the regulation needed.

The Determination of Growth Rate

Observation shows that the growth rate varies between different organs of a developing animal at any one age; between whole animals of the same species, or their organs, at different ages; and between whole animals, or their organs, of different species. The first of these categories, which is responsible for much of the change in shape and proportion that occurs during development, is not open to the simpler kinds of experimental attack, but its very existence reminds us that no systemic growth controlling factor can be expected to operate on all organs equally.

Age differences in growth rate, on the other hand, have been approached, particularly by Twitty (*see* e.g. Twitty 1955), by the method of

transplantation. Contrary to expectation young and rapidly growing eyes transplanted to older animals grew even faster than they would have done if left *in situ*. The decline in the specific growth rate of the eye cannot therefore be due solely to systemic humoral influences. Conversely older and larger eyes grafted into young hosts showed a reduction in growth rate. In both cases the effect was to tend to restore the proper size relation of eye to whole body, and this apparently teleological effect might find an explanation if growth control were sensitive to departures in relative mass of part and whole (*see below*).

Species-specific growth rates are generally, but not always, maintained by organs heteroplastically transplanted. But as Harrison early showed (*see* Twitty 1955) chimeric eyes which start with their separate components (optic cup and lens) of appropriate size for the genetic origin of each, show mutual adjustment towards an eye of compromise size but good functional proportions. Thus, whatever means may serve to adjust organ size to the norm for the species, we have to accept that there may be important but independent mechanisms working within organs to maintain their own components in functional balance.

Pre-functional Organs and Compensatory Growth

There are two main and contrasting views of the control of organ size in the adult. One, which has particular attractions when the organ in question alters the composition of the blood, simply requires that organ growth occurs in response to functional overloading and ceases in its absence. Thus compensatory hypertrophy in a sole surviving kidney, or liver regeneration, could be a response to failure of the remaining tissue to keep blood composition within normal limits, or to the extra work involved in succeeding. This kind of control is unlikely to apply to all organs and has to face difficulties even in the most favourable cases. The major alternative is a development of a theory due to Weiss (1955) which suggests that it is the ratio of mass of organ to mass of organism that is monitored. If so, we might expect that nonfunctional organs could in some circumstances play a part in growth control, and among these are the pre-functional organs of embryo and fœtus (for full discussion *see* Goss 1964).

We may exclude such cases as β-cell activity in the fœtuses of diabetic mothers, and consider only organs that we have reason to believe are not contributing to fœtal life. Few experiments have been reported, but among them those of Viazov *et al.* (1962) deserve repetition. The removal of one maternal lung early in pregnancy of rats appeared to be the cause of excess growth in the fœtal lungs.

Work on amphibia does not suggest that limbs or eyes obey the sort of growth control that is sensitive to the mass of homologous tissue since supernumerary ones grow normally. There is also indirect evidence, for example, that unilateral nephrectomy does not delay the normal atrophy of supernumerary grafted and functionless larval head kidneys (Fox 1960).

Much of the work in this field has come from grafting organs on to the chorio-allantoic membrane of chick embryos. There they share the host's circulation and might deceive it into believing that it has a greater mass of the tissues in question than it should have at its age. Nevertheless, different workers have reported paradoxical results: some find inhibition, some stimulation of growth of the host organ, and some find no change from normal growth. This curious lack of uniformity of results has been paralleled in the effects claimed for tissue homogenates or extracts, variously prepared, upon homologous organs.

The difficulties and confusions still so daunting in this field throw into sharper relief the magnitude and specificity of the action of NGF. It alone might, of course, account for the growth and definitive size of the ganglia sensitive to it, if each ganglionic rudiment were endowed with a finite supply of NGF (as a precursor) which was consumed or otherwise lost during development, and whose exhaustion terminated growth. It is unlikely that any such simple mechanism operates: rather, we must expect that Dr Levi-Montalcini has given us a unique entry into a pattern of growth control which may well operate in the normal development of many vertebrate tissues.

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Meeting March 24 1964
at the Wolfson Institute,
Postgraduate Medical School of London

A symposium was held on **The Measurement of Organ Blood Flow**; the following papers were read:

The Investigation of Limb Blood Flow by Plethysmography
Dr R H Fox

Lung Blood Flow Studies Using Whole Body Plethysmography
Dr Grant de J Lee

Measurement of Segmental Venous Flow by an Indicator Dilution Technique
Dr J Shillingford

Measurement of Hepatic Blood Flow by an Indicator Dilution Technique
Dr Roger Williams
(*see* Williams R, Zimmon D S, Thompson E & Sherlock S (1964) *Gastroenterology* **46**, 525)

Measurement of Renal Blood Flow by a Constant Infusion Indicator Dilution Technique
Dr Geoffrey Walker
(*see* Shaldon S, Higgs B, Chiandussi L, Walker G, Garsenstein M & Ryder J (1962) *J. Lab. clin. Med.* **60**, 954)

Measurement of Venous Flow by Thermal Dilution
Dr R D Lowe

The Electromagnetic Technique for Blood Flow Determination
Dr A Guz

Analogue Computation of Arterial Blood Flow by the Pressure Gradient Method
Dr Ivor Gabe

Regional Pulmonary Blood Flow Measured with Radioactive Gases
Dr J B West

Measurement of Regional Cerebral Blood Flow by Radioactive Tracers
Dr A Murray Harper

Regional Skin Blood Flow Using Radioactive Inert Gases
Dr Graham Bell

Demonstrations were given by: Dr A Guz, Dr M I M Noble, Miss D Trenchard & Dr C A F Joslin; Dr I Gabe; Dr J B West & Dr C T Dollery; Dr R D Lowe & Dr D J Dowsett; Dr B L Pentecost; Mr J T Hobbs